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PERCUTANEOUS ELECTRICAL NERVE FIELD STIMULATION MODULATES CENTRAL PAIN PATHWAYS AND ATTENUATES POST-INFLAMMATORY VISCERAL AND SOMATIC HYPERALGESIA IN RATS

REJI BABYGIRIJA,[a] MANU SOOD,[a] PRADEEP KANNAMPALLI,[b] JYOTI N. SENGUPTA[a,b] AND ADRIAN MIRANDA[a]*

[a] *Department of Pediatrics, Division of Gastroenterology and Hepatology, Medical College of Wisconsin, Milwaukee, WI, United States*

[b] *Department of Medicine, Division of Gastroenterology and Hepatology, Medical College of Wisconsin, Milwaukee, WI, United States*

Abstract—A non-invasive, auricular percutaneous electrical nerve field stimulation (PENFS) has been suggested to modulate central pain pathways. We investigated the effects of BRIDGE® device on the responses of amygdala and lumbar spinal neurons and the development of post-colitis hyperalgesia. Male Sprague–Dawley rats received intracolonic trinitrobenzene sulfonic acid (TNBS) and PENFS on the same day. Control rats had sham devices. The visceromotor response (VMR) to colon distension and paw withdrawal threshold (PWT) was recorded after 7 days. A different group of rats had VMR and PWT at baseline, after TNBS and following PENFS. Extracellular recordings were made from neurons in central nucleus of the amygdala (CeA) or lumbar spinal cord. Baseline firing and responses to compression of the paw were recorded before and after PENFS. Sham-treated rats exhibited a much higher VMR (>30 mmHg) and lower PWT compared to PENFS-treated rats ($p < 0.05$). PENFS decreased the VMR to colon distension and increased the PWT compared to pre-stimulation ($p < 0.05$). PENFS resulted in a 57% decrease in spontaneous firing of the CeA neurons (0.59 ± 0.16 vs control: 1.71 ± 0.32 imp/s). Similarly, the response to somatic stimulation was decreased by 56% (3.6 ± 0.52 vs control: 1.71 ± 0.32 imps/s, $p < 0.05$). Spinal neurons showed a 47% decrease in mean spontaneous firing (4.05 ± 0.65 vs control: 7.7 ± 0.87 imp/s) and response to somatic stimulation (7.62 ± 1.7 vs control: 14.8 ± 2.28 imp/s, $p < 0.05$). PENFS attenuated baseline firing of CeA and spinal neurons which may account for the modulation of pain responses in this model of post-inflammatory visceral and somatic hyperalgesia. © 2017 Published by Elsevier Ltd on behalf of IBRO.

Key words: amygdala, visceral hyperalgesia, colitis, spinal neurons, auricular stimulation.

INTRODUCTION

Recent problems with narcotic dependence and abuse have sparked new ways to think about how to properly manage pain. Improving treatment options and providing alternatives for the treatment of chronic pain in the clinical setting is of critical importance. The challenge primarily lies in avoiding narcotics with a very limited number of treatment options. The development of non-pharmacological or non-addicting approaches to treat or prevent chronic pain is now becoming a major priority. Spinal and deep brain stimulation are an exciting and effective approach that has received much attention (Bittar et al., 2005; Greenwood-Van Meerveld et al., 2005; Lind et al., 2015; Kapural et al., 2016). Unfortunately, due to their invasive nature, they are reserved only for cases of severe, refractory pain. The ability to modulate central pain pathways peripherally, through a non-invasive technique has recently been suggested using the BRIDGE® device (Innovative Health Solutions, Versailles, IN, USA), a FDA-cleared, percutaneous, electrical nerve field stimulator (PENFS) developed to alleviate pain. The device uses specific parameters of stimulation with alternating frequencies to target central pathways.

While the exact mechanism responsible for the analgesic effects is not known, electrical stimulation of peripheral cranial neurovascular bundles in the external ear are believed to help modulate central pain pathways (Ahmed et al., 1998; Sator-Katzenschlager and Michalek-Sauberer, 2007). The external ear in both rats and humans contains branches of four cranial nerves (V, VII, IX, and X) that have projections to brainstem nuclei, particularly the nucleus tractus solitarius (NTS) (Contreras et al., 1982; Folan-Curran et al., 1994; Folan-Curran and Cooke, 2001; Zhang and Ashwell, 2001). The NTS is known to be a "relay station" to other brain structures involved in autonomic control and pain including the rostral ventral medulla (RVM), hypothalamus, amygdala and spinal cord (van der Kooy et al.,

*Corresponding author. Address: Division of Gastroenterology and Hepatology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, United States. Fax: +1-414-456-6361.
E-mail address: amiranda@mcw.edu (A. Miranda).

Abbreviations: CeA, central nucleus of the amygdala; CRD, colorectal distension; NTS, nucleus tractus solitarius; PENFS, percutaneous, electrical nerve field stimulator; PWT, paw withdrawal threshold; RVM, rostral ventral medulla; TNBS, trinitrobenzenesulfonic acid; VMR, visceromotor response.

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1984; Ross et al., 1985; Folan-Curran et al., 1994; Liu et al., 2015). In animals, colonic inflammation has been widely used to investigate the pathogenesis of post-inflammatory pain and as a model for irritable bowel syndrome (IBS) since a large number of patients develop IBS following a gastrointestinal infection (Gschossmann et al., 2002, 2004; Kanazawa and Fukudo, 2014; Löwe et al., 2016). It is not uncommon to see the presence of both visceral and somatic hypersensitivity in the animal models of colitis and in patients with IBS, suggesting a pathway that involves CNS structures (Zhou et al., 2008; Stabell et al., 2013; Patel et al., 2016). Changes in amygdala connectivity and spinal cord processing have been proposed to play a key role in the development of chronic visceral pain (Wang et al., 2013; Qi et al., 2016).

The amygdala is involved in integrating information regarding stress and pain and has been linked to the development of chronic visceral pain in animals and humans (Labus et al., 2009; Johnson et al., 2012; Myers and Greenwood-Van Meerveld, 2012; Rouwette et al., 2012; Wang et al., 2013). Inflammation or pain can cause abnormal activation of the amygdala that could also influence spinal cord processing, since the central nucleus of the amygdala (CeA) projects to brainstem structures and the spinal cord (Burstein and Potrebic, 1993; Saha et al., 2005; Bourbia et al., 2014). Also, primary afferents from the intestine and somatic structures can synapse on the same second-order neurons in the spinal cord (Peles et al., 2004; Lamb et al., 2006). Because of this viscero-somatic convergence, colonic inflammation can influence spinal neurons and higher order structures to produce the phenotype of generalized hyperalgesia (Lamb et al., 2006; Farrell et al., 2016). Overall, however, the exact mechanism leading to post-inflammatory hyperalgesia is not known. To date, there are no studies that investigate the effects of PENFS on amygdala and spinal neurons and the development of post-inflammatory visceral and somatic hyperalgesia.

The objective of the present study was to use an animal model of experimental colitis to investigate the anti-nociceptive properties of PENFS with the BRIDGE device and to explore a central, neuromodulatory mechanism. We hypothesized that PENFS would modulate the response characteristics of amygdala and spinal neurons and prevent the development of visceral and somatic hyperalgesia.

EXPERIMENTAL PROCEDURES

Animals

A total of 61 male Sprague–Dawley (SD) rats weighing 250–300 g were used in this study and data were collected from a total of 47 animals. Animals were housed under conditions of controlled temperature (22–24 °C) and illumination (12-h light cycle starting at 6:00 AM) for at least 7 days before the experiments. Rats were allowed *ad libitum* access to food and water. All experiments were performed according to the approved protocols and guidelines of the Medical College of Wisconsin and The International Association for the Study of Pain and carried out in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals". All efforts were made to minimize animal suffering and to reduce the number of animal in experiments.

Surgical preparation for electrode implantation

Adult rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) as previously described (Mickle et al., 2010). A pair of Teflon coated electrodes (Cooner wire, Part#: A5631) were implanted in the abdominal musculature for EMG recordings. The electrodes were externalized subcutaneously and protected using a silastic tube sutured to the dorsal aspect of the neck. All rats received analgesic (carprofen, 5 mg/kg/day, i.m. for 3 days) and antibiotic (enrofloxacin, 2.5 mg/kg/day, i.m. for 3 days) post-operatively. Following surgery, the animals were housed separately and allowed to recover for at least 5 days prior to further interventions.

Experimental colitis

The rats were fasted for 24 h and then deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.). A 50% solution of TNBS (0.6 ml of 30 mg/ml TNBS in 50% ethanol) was instilled into the colon using a 7-cm-long oral gavage needle inserted into the descending colon. Rats were placed in the supine position with the lower portion of the body slightly elevated in order to prevent leakage of TNBS. The animals were allowed to recover for 5 days prior to further testing.

Measurement of colonic sensitivity

Prior to testing the colonic sensitivity, animals were acclimatized to the experimental conditions by placing them inside a plexiglass-restraining tube (Bollman cage) for two hours a day over 3 days. The visceromotor response (VMR) to colorectal distension (CRD) was used as an objective measure of visceral sensation in all groups as previously described (35). Briefly, individual rats were kept in a Bollman cage while a distensible latex balloon (5 cm in length) attached to PE tubing was inserted into the descending colon and rectum. The opposite end was attached to a distension device. EMG recordings quantified contractions of the abdominal musculature in response to graded CRD. Distention pressures (10, 20, 30, 40, 50, and 60 mmHg) were held constant during the 30-second stimulus with a 180-s, inter-stimulus interval. The EMG signal from the external oblique muscle was amplified through a low noise AC differential amplifier (model-3000: A-M Systems, Inc.) and recorded on-line using the Spike 3/CED 1401 data acquisition program. (CED 1401; Cambridge Electronic Design, Cambridge, UK).

Measurement of somatic sensitivity

Somatic sensitivity was assessed using the paw withdrawal threshold (PWT). The rats were placed on a screen platform and allowed to acclimate to the environment for 20 min prior to testing. Progressive, increasing forces using of Von Frey filaments of various

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